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MEDICINAL PLANTS: AS RESERVOIR OF ANTI- HIV COMPOUNDS FOR HIV/AIDS TREATMENT

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ABSTRACT

According to British medical journal, there are approximately 1.4-1.6 million population with HIV/AIDS. But since last 10 years India has noticed 50% decline in prevalence of HIV/AIDS because of Anti-retroviral therapy. However, resistance of HIV virus to this therapy has also been noticed. In urge to an alternative to this therapy, some medicinal plants were found to have anti-HIV compounds in them. Baicalein and baicalin from *Scutellaria baicalensis* were compounds to have potential anti-HIV activity same as other potential compounds .In order to have detailed study on these natural compounds molecular modelling methods have also been utilized viz. Computer assisted drug design(CADD) by QSAR methods, docking(indirect & direct design) etc.

KEYWORDS: Anti-HIV activity, Anti-HIV compounds, Anti-retroviral therapy, Molecular modelling.

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INTRODUCTION

According to a recent study in British Medical Journal, India has an HIV/AIDS population of approximately 1.4-1.6 million people.[1] According to the United Nations 2011 AIDS report, there has been a 50% decline in the number of new HIV infections in the last 10 years in India.[2]according to the data released by National AIDS Control Organization NACO, India has demonstrated an overall reduction of 57% in estimated annual new human immunodeficiency virus (HIV) infections (among adult population) from 0.274 million in 2000 to 0.116 million in 2011, and the estimated number of people living with hiv was 2.08 million in 2011.[3] The estimated adult HIV prevalence was 0.32% in 2008 and 0.31% in 2009. The states with high HIV prevalence rates include Manipur (1.40%), Andhra Pradesh (0.90%), Mizoram (0.81%), Nagaland (0.78%), Karnataka (0.63%) and Maharashtra (0.55%). The adult HIV prevalence in India is declining from estimated level of 0.41% in 2000 through 0.36% in 2006 to 0.31% in 2009. Adult HIV prevalence at a national level has declined notably in many states, but variations still exist across the states. A decreasing trend is also evident in HIV prevalence among the young population of 15–24 years. The estimated number of new annual HIV infections has declined by more than 50% over the past decade. According to Michel Sidibé, Executive Director of UNAIDS, India's success comes from using an evidence-informed and human rights-based approach that is backed by sustained political leadership and civil society engagement. India must now strive to achieve universal access to HIV prevention, treatment, care and support.[4]

Figure-1
***HIV prevalence in India.*[94]**

The World Health Organization (WHO) recommended that traditional healers be included in national responses to HIV/AIDS.[5] As early as 1989, WHO had already voiced the need to evaluate ethnomedicines for the management of HIV/AIDS: "In this context, there is need to evaluate those elements of traditional medicine, particularly medicinal plants and other natural products that might yield effective and affordable therapeutic

agents. This will require a systematic approach", stated a memorandum of the WHO.[6] Although there are a good number of reports on traditional uses of plants to treat various diseases, knowledge of herbal remedies used to manage HIV/AIDS is scanty, impressionistic and not well documented.[7-9] India has rich plant biodiversity and a long tradition of medicinal use of plants. Several of these plants may contain novel anti-HIV compounds. Thus, it is important to search for novel antiretroviral agents that can be added to or replace the current arsenal of drugs against HIV.[10]

POTENTIAL NATURAL PRODUCTS FROM MEDICINAL PLANTS

Nature has always provided a source of drugs for various ailments. It has reported that several medicinal plants have anti-hiv properties. Crude extracts obtained from bioactivity guided fractionation has provided lead molecules for discovery of potential anti-hiv drug candidates. Natural products possessing anti-hiv potential are shown in Table-1.

Alkaloids: A variety of alkaloids have been found to possess HIV-inhibitory activity. Michellamines are atropisomeric naphthylisoquinoline alkaloid dimers isolated from leaves of *Ancistrocladus korupensis* (family Ancistrocladaceae), a plant native to the Korup National Park in Cameroon's southwest Province. Michellamine B (1) acts both at an early stage of the HIV life cycle by inhibiting reverse transcriptase as well as at later stages by inhibiting cellular fusion and syncytium formation.[11] A tetrahydroxyindolizidine alkaloid, castanospermine (2) isolated from *Castanospermum australe* (family Fabaceae), a plant that occurs naturally in the rainforests of eastern and northern Australia showed inhibition of HIV replication and syncytium formation induced by the envelope glycoprotein of HIV. It has also been reported to have glycosidase inhibitory activity[12]. Buchapine (3), a quinolinone containing two isoprene units and its structural isomer 3-(3-methyl- 2-butenyl)-4-[(3-methyl-2-butenyl) oxy]-2(1H)-quinolinone (4) isolated from *Eodia roxburghiana*, a plant indigenous to Southeast Asia and Australia, protected CEM-SS cells from the cytopathic effects of HIV-1 *in vitro*.[13] Sesquiterpene pyridine alkaloids, triptonine A (5), triptonine B (6) and hypoglaunine B (7) isolated from *Tripterygium hypoglaucum* and *T. wilfordii* exhibited potent *in vitro* anti-HIV activity with a therapeutic index of more than 1000.[14] FK-3000 (8), a morphine-related compound obtained from methanolic extract of root tubers of *Stephania cepharantha* (family Menispermaceae), inhibited the cytopathic effects of HIV-1 on MT-4 cells at 7.8 µg/ml. Another alkaloid, cepharanthine (9) isolated from the same plant, has been reported to have antiallergic, anti-inflammatory and immunomodulatory activity and also can potently inhibit HIV-1 replication.[15] Nitidine (10), isolated from roots of *Toddalia asiatica* (family Rutaceae), showed significant anti-HIV activity in the cell-based assay. It is also reported to have HIV-reverse transcriptase inhibitory activity.[16] *O*-Demethyl buchenavianine (11), a piperidine-flavonerelated alkaloid isolated from *Buchenavia capitata* (family Combretaceae), showed activity in both anti-HIV and anticancer cell-based screens.[17] Harmine (12) isolated from *Symplocos setchuensis* was found to inhibit HIV replication in H9 lymphocyte cells. Amongst its 28 derivatives, *N*butylharmine (13) was found to be most potent with EC50 of 0.037 µM and therapeutic index of 210.[18] 1-Methoxy canthinone (14) isolated from *Leitneria floridana*, showed potent anti-HIV activity (EC50 is 0.26 µg/ml).[19]

Coumarins: Coumarins such as calanolides and inophyllums have been established as non-nucleoside-specific inhibitors of HIV reverse transcriptase. These are obtained from various species of *Callophyllum* (family Clusiaceae), the genus primarily found in the Indo-Pacific region, particularly Malaysia.[20] (+)-Calanolide A (15), (–)-calanolide B (16) and its dihydro-derivative, (–)-7,8-dihydrocalanolide B isolated from the fruits and twigs of *C. lanigerum*, significantly inhibited the cytopathic effects of HIV-1 in T-cell lines, including both CEM-SS cells and MT-2 cells. All three calanolides inhibited the laboratory-adapted HIV-1 variants, the clinical viral isolates,

inclusive of the diverse clades (A–F), syncytium-inducing and non-syncytiuminducing isolates, and T-tropic and monocyte-tropic isolates.[21] Sarawak MediChem Pharmaceuticals, Malaysia has the exclusive worldwide license to the calanolide class of compounds from the National Cancer Institute. They have successfully completed early phase I/II 48-subject clinical trial of calanolide A in combination therapy for HIV, which evaluated the effect of therapy on pharmacokinetic enhancement and safety. Results of the trial confirmed that the combination therapy was effective in increasing the blood levels of calanolide in human volunteers. Additionally, no serious adverse events were noted in any subjects and the small number of adverse events observed was similar to those previously associated with the drug. Calanolide A is currently in phase II clinical trials, focused on assessment of its long-term anti-HIV activity in combination with other anti-HIV agents and an assessment of the long-term durability of such drug combinations.[22] Cordatolide A (17) and B (18), structural analogues of calanolides isolated from *Callophyllum cordato-oblongum* showed potent inhibitory activity against HIV-1 replication in a novel green fluorescent protein-based reporter cell assay.[23]

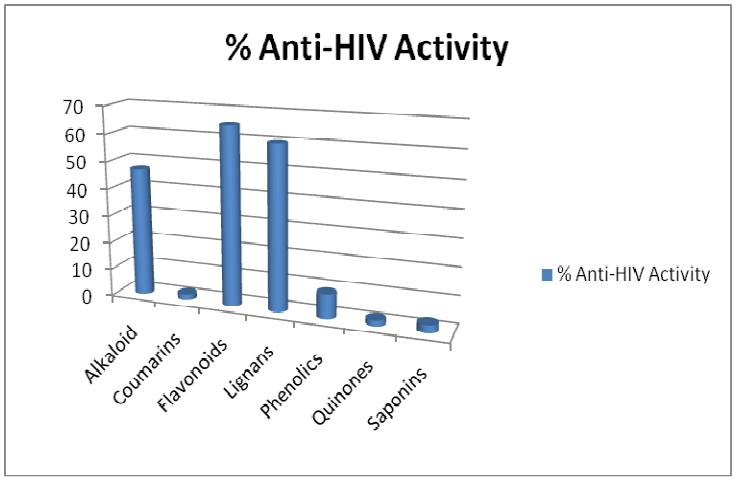

Figure-2
***% Anti-HIV activity in different natural compounds.*[93]**

Table-1: Anti-HIV natural products

Natural product	Source	Anti-HIV activity	References
Alkaloids			
Batzelladines A (102)	*Batzella* sp.	10 µM	83
Batzelladines B (103)	*Batzella* sp.	25 µM	83
Buchapine (3)	*Eodia roxburghiana*	0.94 µM	84
Castanospermine (2)	*Castanospermum austral*	> 10 µg/ml	85
Coumarins			
(+)-Calanolide A (15)	*Callophyllum lanigerum*	0.2 µM	86
(–)-Calanolide B (16)	*C. lanigerum*	0.2 µM	86
Flavonoids			
Hinokiflavone (29)	*Rhus succedanea*	65 µM	87
Robustaflavone (28)	*R. succedanea*	65 µM	87
Lignans			
Anolignan A (32)	*Anogeissus acuminata*	60.4 µg/ml	88
Anolignan B (33)	*A. acuminata*	1072 µg/ml	88
Phenolics			
Balanocarpol (60)	*Hopea malibato*	*	89
Camellia tannin H	*Camellia japonica*	0.9 µM	90
Quinones			
Conocurvone (63)	*Conospermum incurvum*	0.02 µM	91
Hypericin (64)	*Hypericum perforatum*	*e	92
Saponins			
Actein (65)	*Cimicifuga racemosa*	0.375 µg/ml	93

IMPACT OF MEDICINAL HERB ON ANTIRETROVIRAL DRUG THERAPY

Current therapy against AIDS is based on six classes of anti-HIV drugs: the nucleoside and nucleotide reverse transcriptase (RT) inhibitors (NRTIs and NtRTIs), the non-nucleoside reverse transcriptase inhibitors (NNRTIs), the protease inhibitors (PIs), the fusion inhibitor (FIs), the entry inhibitors and HIV-1 integrase inhibitors.[95] However, medicinal plants are gaining popularity because of several advantages such as fewer side-effects, better patient compliance, relatively low cost and high accessibility as well as high acceptability due to a long history of use.[24] Traditional herbal medicines that formed the basis of health care since the earliest of days are still widely used. In developing countries, traditional herbal medicines are often used in great numbers with an application of indigenous knowledge. Since traditional healers are spread throughout rural areas in developing countries and there is often lack of an efficient primary health care system, they are widely consulted by human immunodeficiency virus (HIV) infected patients of whom some may already have developed acquired immunodeficiency Traditional herbal medicines are the main *materia medica* of traditional healers, however, HIV/AIDS patients may simultaneously also take Western drug therapies provided by clinics and hospitals. The combined use of traditional herbal medicines with antiretroviral drugs could potentially cause drug–herbal pharmacokinetic and/or pharmacodynamic interactions. Potential pharmacokinetic interactions include changes in the absorption of the co-administered drug, thereby influencing its bioavailability and consequently its therapeutic effectiveness or side-effects. One interaction that may influence drug absorption is interference of the co-administered medicinal plant with the active transport process during efflux.[25-27]

Effluxmechanisms such as P-glycoprotein (P-gp) are responsible for transporting a broad range of compounds out of the intestinal epithelial cells back into the intestinal lumen. Several reports have indicated that P-gp plays an important role in oral drug absorption.[28-29] P-gp is a transmembrane protein with amolecularweight of 170 kDa and is located among other sites, on the apical membrane of the enterocytes of the intestinal epithelium. Three isoforms of P-gp have been identified in rodents (Class I, II and III) and two in humans (Class I and II). Class I- and II-glycoproteins are known to exhibit multidrug resistance, whereas class III-glycoprotein does not exhibit this characteristic.[30-31] Furthermore, its expression and functions are increased with human ageing and with HIV-1 infection.[32-33] Inhibition of P-gpmay lead to enhanced absorption of drugs that are substrates for this active transporter.[34] Four classes of orally administrated antiretroviral agents are currently available for the treatment of HIV infection, which include nucleoside reverse transcriptase inhibitors, e.g. zidovudine, lamivudine, zalcitabine, didanosine, tenofovir, stavudine, abacavir; non-nucleoside reverse transcriptase inhibitors, e.g. nevirapine, delavirdine, efavirenz; protease inhibitors, e.g. saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir, atazanavir and fusion inhibitors, e.g. enfuvirtide. There have been several studies demonstrating that protease inhibitors are substrates for P-gp, which can limit their intestinal absorption and their transport across the blood–brain barrier.[35,30,36] This creates considerable potential for pharmacokinetic drug interactions when concomitantly administrated with other compounds that act as inhibitors of P-gp.[30,37]

Several medical claims have been made regarding the use of *Sutherlandia frutescens* (L.) R.Br. [syn. *Lessertia frutescens* (L.) Goldblatt & J.C. Manning] (Fabaceae), common name “cancer bush” such as improvement of the quality of life of HIV/AIDS patients by combating weight loss and regaining of energy and appetite. This plant, which grows wild in the Western Cape and in the hills of Zululand in South Africa, has been used for centuries by indigenous people including the San for the treatment of different illnesses. Evidence exists that it has been used for treatment of cancer, tuberculosis, diabetes, chronic fatigue syndrome, influenza, rheumatoid arthritis, osteoarthritis, peptic ulcers, gastritis, reflux esophagitis, menopausal symptoms, anxiety and clinical depression. A principle ingredient in *Sutherlandia frutescens* is l-canavanine, a non-

protein amino-acid and structural analogue of arginine, which has been reported to have anti-viral activity and anti-tumour properties.[38-39] One of the applications of *Hypoxis hemerocallidea* Fisch. & C.A. Mey. (Hypoxidaceae), common name "African potato" corms is to boost the immune systems of patients suffering from HIV/AIDS, but they are also used for the treatment of the common cold, flu, arthritis and cancer. This plant grows wild in Kwa-Zulu Natal and Pondoland in South Africa and has been used by Zulu traditional healers for the treatment of urinary tract infections, heart weakness, internal tumours and nervous disorders. Sterols and sterolins are some of the active ingredients identified in this medicinal plant.[40-41]

POTENTIALITY OF DIFFERENT COMPOUNDS

Baicalein and baicalin. Ono *et al.* showed the effects of baicalein on the activity of various reverse transcriptases. They demonstrated that 1 µg/ml baicalein inhibited 90% of the activity of MLV-reverse transcriptase, and that 2 µg/ml baicalein inhibited 90% of the activity of HIV-reverse transcriptases.[42] Tang[43] found that baicalin, which is isolated from *Scutellaria baicalensis* Georgi, inhibited HIV-reverse transcriptase, with an IC50 value of 22 µM. Some pharmacological test results have demonstrated noncompetitive, inhibition of retroviral reverse transcriptase activity in HIV-1-infected H9 cells[44], HIV-1 specific core antigen p24 expression, and quantitative focal syncytium formation on CEM-SS monolayer cells.[45] Baicalin, and its derivative 7-glucuronic acid 5, 6- dihyorxyflavone were also efficacious in inhibiting reverse transcriptase of other retroviruses.[46] The difference in HIV-1 reverse transcriptase inhibitory activity between baicalein and baicalin has been examined.[47] The results show that the HIV-1 reverse transcriptase inhibitory activity of baicalein was four times higher than baicalin. The inhibition of HIV-1 integration (Step 5 of replicative cycle) by baicalein was investigated biochemically and by means of structure-activity relationships. It was reported that IC50 for HIV integrase inhibition by baicalein was 4.3 µM.[48] An investigation on the metabolism of baicalin has been published.[49] The results indicated that baicalin was first metabolized into baicalein, and the final metabolite was identified as baicalein 6-*O*-sulfate by comparing its retention time in highperformance liquid chromatography (HPLC), and electrospray ionization mass spectra (ESI-MS)/MS methods with that of an authentic sample.

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MECHANISMS OF ACTION

As for the mechanism of the anti-HIV-1 effect of baicalin, it was found that baicalin and baicalein have an inhibitory effect on various cellular DNA and RNA polymerases [50-51]. In the case of baicalein, the mode of inhibition was of the competitive type (murine leukemia virus reverse transcriptase and HIV-1 reverse transcriptase) with respect to the template primer ((rA)n(dT) 12–18), or mixed type suggesting that baicalein also inhibits HIV-1 reverse transcriptase activity by interfering with the binding of viral RNA to the reverse transcriptase molecule near the active site of the enzyme. Baicalin does not inhibit the activity of HIV-2 reverse transcriptase, or murine leukemia virus reverse transcriptase. Futhermore, baicalin neither inhibited the binding of OKT4A mAb to the gp 120 binding site of CD4, nor interfered with the gp 120-CD4 binding. This definitely rules out the possibility that baicalin interferes with the virus adsorption step (Step 1 of replicative cycle). Flavonoids such as gardennin, myricetin, and baicalein were found to inhibit HIV-1 protease. However, the IC50 value of baicalein was 480 µM, almost 44 times that of gardennin (IC50 = 11 µM).[52]

EFFICACY OF HERBAL FORMULATION

As mentioned above, Xiao-Chai-Hu-Tang or Sho-saikoto consists of a mixture of aqueous extracts from seven different plants. Seven and a half grams of this contains 4.5 g of dried extract, which is prepared from boiled water extracts of seven herbs: 7.0 g of *Bupleurum* root, 5.0 g of *Pinellia* tuber, 3.0 g of *Scutellaria* root, 3.0 g of *Jujube* fruit, 3.0 g of *Ginseng* root, 2.0 g of *Glycyrrhiza* root, and 1.0 g of *ginger* rhizome.[53-54] Some research groups demonstrated that among the active components of Sho-saiko-to, baicalein and baicalin were found to be mainly responsible for antioxidative[55], anti-tumor , anti-proliferative

[62,42,63], and anti-HIV activity. It is interesting to note that data on antioxidative activity between Sho-saiko-to and *Scutellaria* root using MeOH extracts were very similar.[53] Our group and other researchers indicated that the water extracts of *Scutellaria* root also have significant antioxidant activity.[56-65] In the four major constituents, the order of antioxidant activity is baicalein , baicalin, worgonin ,wogonoside.[66] Antioxidant and other mechanisms may also play a role in the anti-HIV effects of baicalin and baicalein. An oral dose toxicity study of Sho-saiko-to in rats has been reported.[67] Two oral doses (2 and 6.4 g/kg) of Sho-saiko-to were administered to the animal after overnight fasting, and no death was observed.

MOLECULAR MODELING METHODS FOR ANTI-AIDS COMPOUNDS

1] Computer-Assisted Drug Design and Molecular Modeling

Depending on the lead compound used to develop new, anti-AIDS compounds, an important method that can be applied is computer-assisted drug design (CADD). The earliest method has been called quantitative structure-activity relationships (QSAR). The currently used method involves traditional or classic QSAR and 3D QSAR. In the traditional approach to QSAR, the chemical structure can be described with experimental and theoretical steric, electronic, and hydrophobic parameters. 3D QSAR methods were developed as an alternative to traditional QSAR to describe molecules more "realistically", i.e., with properties of molecules calculated from their three-dimensional structures. These two approaches of QSAR are widely used in the area of drug design and agrochemistry design. Garg *et al.* summarized the investigations with QSAR for anti-HIV drug design. The relationship between structure and anti-HIV activities, log *P* volume (partition coefficient) and stereo effect are very important parameters.[68] A3DQSAR research set of 15 flavones, including baicalein, that inhibit HIV-1 integrase-mediated cleavage and integration *in vitro* were tested using Comparative Molecular Field Analysis (CoMFA). The results show a strong correlation between the inhibitory activity of these flavones and the steric and electrostatic fields around them. A diversity analysis of 14156 molecules tested by the National Cancer Institute for anti-HIV activity using the quantitative structure-activity relational expert system MCASE has been reported. This study shows that certain structure-activity relationships exist among the anti-HIV-1 agents. They found that log *P* and the Highest Occupied Molecular Orbital (HOMO) coefficient of hydrogen bond acceptors are important factors for the activity of some biophores. With the help of the resulting model, they have tested 10highly diverse chemicals that came from different sources, the overall accuracy of their prediction being 80%. This result provides a first glance at the possible predictivity of MCASE.[69]

Molecular modeling has become a well-established research area during the last decade due to advances in computer hardware and software that have brought high performance computing and graphics within the reach of most academic and industrial laboratories. It is very important to realize what is really meant by "computer-assisted drug design" (CADD) with the QSAR method. Molecular modeling systems provide powerful tools for building, visualizing, analyzing and storing models of complex molecular systems (i.e. inhibitor binding with receptor) that can help interpret structure-activity relationships.[70] There are the two major molecular modeling strategies currently used in the conception of new drugs for macromolecules (called "direct design") and small molecules (called "indirect design"). In direct design, the three-dimensional features of a known receptor site are directly considered. Indirect design is based on the comparative analysis of the structural features of known active and inactive molecules that are interpreted in terms of complementarity with a hypothetical receptor site model. Over the past 15 years, molecular modeling combined with database searching has become a part of the drug discovery and design process. An increasing number of applications for molecular modeling combined with database searching has led to the discovery of new lead compounds used in drug design.[71-72] Database searching programs have been developed and are being widely used in an integrated fashion with molecular modeling systems such as DOCK, which

depicts a small molecule docking to the macromolecule.[73-74] DOCK is typically used to generate proposed binding orientations of small molecules, (ligands, such as an inhibitor) and macromolecules, (receptor, such as an enzyme) with an X-ray crystallographic structure of the macromolecule as the starting point. DOCK can find many orientations for a single molecule, or it can be used to search a database to identify compounds that may bind with a macromolecular site. The output compounds from DOCK are uniformly oriented in the target site and can be viewed by most molecular modeling programs.[75]

2] Molecular Modeling in Anti-HIV Studies

The application of molecular modeling to develop new anti-HIV drugs is just unfolding . The discovery of a novel, non-peptide, HIV-1 protease inhibitor has been reported [76]. Fifteen novel, non-peptide HIV-1 protease inhibitors were identified by flexible 3D database pharmacophore searching of the National Cancer Institute Drug Information System (DIS) database. The pharmacophore query used in the search was derived directly from the X-ray-determined structures of protease/inhibitor complexes.[77-79] These 15 inhibitors, belonging to nine different chemical classes, are promising leads for further development. The two best inhibitors found, NSC 32180 and NSC 117027, had IC50 values of 0.32 and 0.75 μM, respectively, for HIV-1 protease inhibition.[80-82]

CONCLUSION

Plants are an important source of anti-HIV chemical compounds, and A large number of plants contain anti-hiv potential that could be used to develop new drugs to treat hiv/aids. Therefore, Current information provides an important contribution to our understanding of plants that can be used in the treatment of hiv/aids. This contribution should help further research and public intrest to isolate newer nti-hiv potential plants.

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